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The World Reference Center for Arboviruses received viruses from the United States and foreign countries for characterization and identification. New viruses, or viruses causing diseases not previously recognized included Sabia virus, a new arenavirus causing fatal hemorrhagic fever in Sao Paulo State, Brazil. Another arenavirus, Guanarito causes Venezuelan hemorrhagic fever, and was newly characterized. Venezuelan equine encephalitis, variety 1E, was for the first time recognized as epizootic in horses of Chiapas, Mexico. The confirmation of Rift Valley fever was made in Egypt during the return in 1993 of this disease of humans and domestic animals after an absence of 13 years. Recombinant vaccinia viruses were constructed to express proteins of, Japanese encephalitis (JE) and the four serotypes of dengue (DEN). Pigs were protected against viremia by a recombinant expressing JE preM/E as were mice by recombinants expressing YF and DEN preM/E. The low passage collection of arboviruses was augmented, and reference reagents were distributed to scientists in 21 countries.

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FOREWORD

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RES Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

RES In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

RES For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

RES In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

Robert E. Shope May 29, 1984

PI Signature Date

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INTRODUCTION

The World Reference Center for Arboviruses was established at the Yale Arbovirus Research Unit in 1965 as an outgrowth of The Rockefeller Foundation program on arboviruses which was moved in 1965 to Yale University from New York City. The U.S. Army has supported this program since 1972, initially through joint Navy-Army funding, then through separate contracts and grants, and during the past 3 1/2 years by this grant. The progress of the past 21 months is included in this report; it covers the work for the entire project which received support from the World Health Organization and the U.S. National Institutes of Health in addition to this grant.

BODY OF REPORT

I. Identification of Sabia virus, a new arenavirus causing hemorrhagic fever in Sao Paulo State, Brazil (R. Tesh, R. Rico-Hesse, J.-P. Gonzalez, and R. Shope, in collaboration with A. Travassos da Rosa, Instituto Evandro Chagas, Belem, Brazil; L. Souza, Instituto Adolfo Lutz, Sao Paulo, Brazil; and P. Jahrling, USAMRIID, Fort Detrick)

Sabia virus, SP H-114202, was isolated from a case of hemorrhagic fever by the staff of the Instituto Adolfo Lutz from blood collected January 16, 1990, the day of death. The patient was a 25 year old white female who was trained as an agricultural engineer. She had visited Rondonia for two weeks in September 1989, 4 months prior to her illness. Otherwise she had not been outside of Sao Paulo State. The virus was identified in Brazil during 1992 as an arenavirus by staff of the Instituto Evandro Chagas and was submitted for further study to Yale University. During September 1992, a senior technician at the Instituto Evandro Chagas became ill while working with Sabia virus, and virus was isolated from his blood. He survived without sequellae.

At Yale, SP H-114202 virus was passaged in Vero E6 cells and lyophilized for stock. Only limited further passages were made to obtain RNA and for IFA. IFA studies confirmed that SP H-114202 was an arenavirus. The lyophilized virus was then provided to CDC and USAMRIID, Fort Detrick for continuing studies under BSL-4 conditions. The Unit also supplied reference antigens and antibodies of a battery of New World arenaviruses to the Instituto Evandro Chagas where complement fixation tests demonstrated that Sabia virus was distinct from all known South American arenaviruses. Plaque neutralization tests at USAMRIID also indicated that Sabia was new to science.

In molecular studies, 250 nucleotides from the 3′ end of the S segment of Sabia virus RNA were compared to those of 5 other Tacaribe complex viruses, Junin, Machupo, Guanarito, Tacaribe and Pichinde. Limited sequence analysis showed that Sabia virus was 30% divergent from Junin, Machupo and Guanarito viruses, the 3 other New World arenaviruses causing hemorrhagic fever; it was most closely related to Pichinde, a virus isolated from rodents in southwestern Colombia. These results confirmed that Sabia virus was distinct from the other human pathogenic viruses in the Tacaribe complex and suggest that it has been circulating in an isolated and still unknown ecologic niche for a long time.

II. Identification of variety 1E Venezuelan encephalomyelitis (VE) virus associated with an equine epizootic in Chiapas, Mexico (R. Rico-Hesse in collaboration with P. Fernandez, USDA)

An epizootic of encephalitis in horses in Chiapas, Mexico was reported in June 1993. The epizootic involved 97 of 417 horses in the coastal area. Forty-three horses died and VE virus was isolated at the laboratory of the joint Mexico-U.S. Exotic Animal Disease Commission from the brain of one of the horses. The last epizootic of VE in Mexico was in 1971 when 6,000 equines died and many humans were also affected. Vaccination was practiced over the 20 year period, but was discontinued in March 1991 by the Government which declared Mexico free of epizootic VEE virus in June 1992.

The isolate from the horse brain was submitted to Yale for study. Monoclonal antibody reactivity pattern indicated that the strain was variety 1E which is the local Mexican enzootic variety, not previously known to cause epizootics in horses. The reason that a previously endemic VE virus became epizootic is not known; the working hypothesis is that a mutation in the 1E variety has conferred equine virulence.

III. Recurrence of Rift Valley fever in Egypt after an absence of 13 years (R. Shope in collaboration with R. Arthur, NAMRU-3, Cairo, Egypt).

Rift Valley fever (RVF) was diagnosed in human cases of ocular disease in Egypt during May 1993. The infection had not been recognized in Egypt for 13 years and was presumably reintroduced. Tests at Yale of 3 human sera sent by NAMRU-3 confirmed the presence of RVF specific IgM in the sera. The results were as follows:

Serum number	3	7	8
RVF total Ig	1:3200	1:12800	1:6400
Sicilian SF total Ig	<1:200	1:800	<1:200
Naples SF total Ig	1:400	<1:200	<1:200
RVF IgM	1:6400	1:6400	1:6400

IV. Venezuelan hemorrhagic fever field studies implicating rodents in the epidemiology of Guanarito virus in Venezuela (R. Tesh and M. Wilson in collaboration with R. Salas, National Institute of Hygiene, Caracas, Venezuela, and with C. Peters and T. Ksiazek, CDC).

In the previous annual report, the discovery of a new arenavirus causing Venezuelan hemorrhagic fever (VHF) was reported along with its serological characterization as a newly recognized agent. Reasoning by analogy with other arenaviruses, it was postulated to be transmitted to people from a rodent reservoir. Supplementary funds from the NIH to the Reference Center Grant, enabled Yale staff to collect rodents in Venezuela in collaboration with scientists of the National Institute of Hygiene.

During 3 weeks in February 1992, small mammals were trapped in 4 different rural communities, La Hoyada, Palmarito, La Arenosa, and Pirital, in or adjacent to the Municipality of Guanarito, Portugesa State, northwestern Venezuela. Cases of VHF had occurred in each of the 4 communities. The communities are rural comprising a patchwork of savanna, gallery forest, and cultivated lands. Most of the population is involved in agriculture and cattle raising. An attempt was made to sample rodents in a variety of habitats. The rodents were trapped alive, then sacrificed to collect heart blood and spleen. Samples were tested for virus and antibody at CDC. In addition venous blood samples were also collected from 195 healthy people from La Hoyada, where seven cases of VHF had occurred since October 1989. The subjects ranged in age from 2 to 90 years.

Two hundred fifty-two small mammals, representing 11 species, were collected. The animals collected and the viruses isolated are shown in Table 1. Thirty-one of the 252 animals yielded Guanarito virus. Forty-seven per cent of the cotton rats (Sigmodon alstoni were positive, as were 11.3 % of the cane mice (Zygodontomys brevicauda. Other rodents and marsupials were negative.

Bloods from 223 of the rodents were tested by IFAT. Antibodies to Guanarito virus were detected in 5 different rodent species as shown in Table 2. The highest prevalence was in the cane mice (15%), whereas the cotton rat had 5.1%. Nine of hte 12 cane mice that yeilded Guuanarito virus from their spleens also had antibodies to the virus in their sera. In contrast, none of the 19 virus-positive cotton rats had Guanarito antibody.

Sera from 195 healthy residents of La Hoyada were tested by IFAT and IgG ELISA. Five of these had antibody by both tests. This was the criterion for considering human sera positive. Therefore the point prevalence in 1992 was 2.6%. All positive samples were from adults, one male and 4 female. Two of the 5 had been previously diagnosed clinically as VHF.

These results implicate the cotton rat as the reservoir and suggest that the inapparent to apparent infection ratio in people may be as low as 3 to 1.

V. Serosurvey of Egyptians living in a flooded village of the Nile Delta (R. Shope in collaboration with M. Darwish of Ain Shams University, Cairo)

A serosurvey for arbovirus antibodies was carried out in inhabitants of a flooded village, Begiram, in Minufiya Governorate, 70 km north of Cairo. This village is representative of an established Nile Delta rural farming community. Its population is about 6,500 and the inhabitants are nearly all life-long residents with very little travel.

Water seepage was first noted about 10 years ago and is still continuing. The underlying factors behind the inundation are not well understood, and no effective control measures have been taken.

Table 1. Guanarito virus isolates from spleens of wild animals captured at four localities in Portuguesa State, Venezuela in February 1992*

Animal	Locality				Total
	La Hoyada	Palmarito	La Arenosa	Pirital	
Zygodontomys brevicauda	6/52	3/5	3/46	0/3	12/106
Oligoryzomys fulvescens	0/11	-	0/11	-	0/22
Sigmodon alstoni	11/21	0/1	8/17	0/1	19/40
Holochilus brasiliensis	0/1	-	0/2	-	0/3
Heteromys anomalus	-	-	0/1	-	0/1
Oecomys flavicans	-	0/1	-	-	0/1
Oecomys speciosus	0/2	-	-	-	0/2
Proechimys guairae	0/13	0/5	0/5	0/2	0/25
Rattus rattus	0/20	0/9	0/5	-	0/34
Marmosa robinsoni	0/11	0/2	0/2	0/1	0/16
Didelphis marsupialis	-	0/1	-	0/1	0/2

*Values are the number of positive/number tested. - = not tested.

Table 2. Prevalence of antibodies to Guanarito virus in sera of rodents captured at four localities in Portuguesa State, Venezuela in February 1992*

Animal	Locality				Total
	La Hoyada	Palmarito	La Arenosa	Pirital	
Zygodontomys brevicauda	6/49	3/5	6/43	0/3	15/100
Oligoryzomys fulvescens	0/11	-	2/10	-	2/21
Sigmodon alstoni	2/20	0/1	0/17	0/1	2/39
Holochilus brasiliensis	0/1	-	0/2	-	0/3
Heteromys anomalus	-	-	0/1	-	0/1
Oecomys flavicans	-	0/1	-	-	0/1
Oecomys speciosus	0/2	-	-	-	0/2
Proechimys guairae	1/13	0/4	0/5	0/2	1/24
Rattus rattus	0/91	0/9	2/4	-	2/32

*Values are the number of positive/number tested. - = not tested. Sera were screened at an approximate dilution of 1:20 by IFA with Guanarito antigen.

During the past decade some large and numerous small pools of stagnant water were created in the lowest-lying section of the village. Water consumption increased as the population grew and as people gained easy access to water piped to the homes. There was no waste water and sewage disposal system. As a result, the water accumulations were heavily polluted and offered favorable conditions for breeding of Culex mosquitoes, major vectors of arboviruses in the Nile Delta. The people complained of large numbers of mosquitoes, and also of increasing prevalence of summertime fevers in all age groups. For this reason, the present study was designed to determine baseline levels of antibodies to Sindbis, West Nile, and Rift Valley fever. Naples, Sicilian, Quaranfil, Crimean-Congo hemorrhagic fever, and Hantaan viruses were also tested. Serology was done by ELISA except for Hantaan which was done by IFA. Sera were screened at the 1:50 dilution, then positive sera were titered.

Table 3 shows the prevalence by age group of antibodies to West Nile, Rift Valley fever, Sicilian, and Naples sand fly fevers. West Nile antibody prevalence was 46% with an increase in antibody with increasing age. Nearly 100% of individuals 50 years and older had antibody. Sicilian sandfly fever antibody prevalence was also 45%, however the pattern was very different from West Nile; the percentage was nearly the same irrespective of age group. Naples sand fly fever virus prevalence was 21% with rising percentages in the younger age groups, plateauing at 20 years and above. Twenty-five sera were positive for both Sicilian and Naples (Table 4), which are related to each other. Of these, 9 were equal or greater than 4-fold higher titer to the Sicilian virus and 8 were equal or greater than 4-fold higher titer to the Naples virus. The remaining 8 of 25 were nearly equal in titer to the Sicilian and Naples viruses and were considered probable double infections.

Rift Valley fever antibody was detected in 5% of those surveyed, and was absent from individuals under 20 years of age.

Antibodies to the tick-borne viruses Quaranfil and Crimean-Congo hemorrhagic fever was detected in only 5 and 2 sera respectively of the 180 tested. Hantaan abtibody was found in 2 sera also. There were no evidences of Sindbis and Batai viruses. Hantaan antibody was found in 2 sera also. There were no antibodies to Sindbis and Batai viruses.

The antibody prevalence to West Nile was more than double the prevalence in 1991 Bilbeis, a neighboring village, consistent (but not proof of) the hypothesis that high Culex activity was present. The Rift Valley fever antibody was entirely in persons over 20 years of age, indicating that the recent recurrence of Rift Valley fever developed after these sera were taken. The pattern of Naples and Sicilian sand fly fever antibodies, i.e. rising levels in young people, then a plateau in persons over 10 or 20 years old, is characteristic of a virus that is very focal, immunizes nearly all persons at risk at an early age, but does not spread to persons outside of the focus. This pattern may reflect the very focal nature of the vector, Phlebotomus papataci.

Table 3

Antibody prevalence of West Nile, Rift Valley fever (RVF), Sicilian sandfly fever (SF), and Naples sandfly fever viruses in inhabitants of Begiram, Egypt

Age	Number	West Nile		RVF		Sicilian SF		Naples SF	
		Positive	%	Positive	%	Positive	%	Positive	%
0- 9	16	1	6	0	0	7	44	1	6
10-19	52	9	17	0	0	23	44	5	10
20-29	26	9	35	4	15	10	39	9	35
30-39	35	18	51	4	11	17	49	8	23
40-49	24	18	75	0	0	11	46	8	33
50-59	15	15	100	1	7	7	47	4	27
60-above	12	12	100	0	0	7	58	3	25
TOTAL	180	82	46	9	5	82	46	38	21

Table 4

Antibody titers in 25 sera reactive with both Sicilian sandfly fever and Naples sandfly fever viruses by ELISA

Sicilian	Naples	Probable infection
1:3200	1:50	Sicilian
1:1600	1:100	Sicilian
1:3200	1:100	Sicilian
1:1600	1:100	Sicilian
1:6400	1:1600	Sicilian
1:800	1:50	Sicilian
1:1600	1:100	Sicilian
1:6400	1:800	Sicilian
1:1600	1:400	Sicilian
1:200	1:3200	Naples
1:50	1:1600	Naples
1:800	1:3200	Naples
1:100	1:1600	Naples
1:800	1:1600	Naples
1:100	1:3200	Naples
1:800	1:6400	Naples
1:400	1:1600	Naples
1:100	1:100	Sicilian & Naples
1:1600	1:3200	Sicilian & Naples
1:100	1:50	Sicilian & Naples
1:1600	1:1600	Sicilian & Naples
1:100	1:200	Sicilian & Naples
1:800	1:1600	Sicilian & Naples
1:800	1:800	Sicilian & Naples
1:800	1:1600	Sicilian & Naples

VI. Studies of recombinant vaccinia viruses expressing flavivirus antigens (P. Mason, R. Shope, B. Fonseca, and E. Konishi in collaboration with E. Paoletti)

a. Requirement of preM for maturation of Japanese encephalitis virus. This study was undertaken as part of the Unit's efforts to understand replication of flaviviruses with a longer term aim of producing vaccines for Japanese encephalitis and dengue viruses. Recombinant vaccinia viruses were used to produce authentic Japanese encephalitis proteins in HeLa cells. In prior Annual Reports we described two of these viruses, vP555 and vP829, that encoded prM and E and induced the proper synthesis of both intracellular and extracellular forms of the prM/M and E proteins in HeLa cells, in the presence (vP555) or absence (vP829) of NS1. Also, cells infected with vP829 released subviral particles of 20-nm diameter that were characterized as flavivirus RNA-free membrane vesicles with prM/M and E embeddeed in a lipid bilayer. Another recombinant, vP658, encoded E and NS1, and E produced by cells infected with this virus was not released into the extracellular fluid.

In the present studies, HeLa cells were infected with vP658 (E not released from cells) and with vP829 (E released). Indirect immunofluorescopy of these cells stained with MAb against E showed that both recombinants induced a fine network in the cytoplasm, suggesting that E produced by either virus is present in the ER.

The reactions of E protein in cells infected with vP658 and vP829 viruses were determined by reacting cells with a panel of MAbs. Radioactive antigens (35S methionine) prepared from cells infected with the Nakayama strain of JEV, vP829, or vP658 were immunoprecipitated with MAbs and subjected to SDS-PAGE, and the amounts of E precipitated were determined by using a Molecular Dynamics PhosphorImager. The following were the results obtained with three of the MAbs:

Virus	Relative amount after precipitation with MAb and SDS-PAGE analyses		
	J3-11G5	J3-14E6	D1-4G2
JEV	57	35	39
vP829	95	60	55
vP658	9	6	1

J3-11G5 was selected because it was the MAb that reacted to the highest degree with JEV, and J3-14E6 because it reacted least with JEV. D1-4G2 MAb was known to react with a discontinuous epitope and its binding is dependent on correct disulfide bond formation within E. The results indicated that the vP658-encoded E was present in cell lysates in much lower amounts, probably as a result of degradation since identical vectors and promoters were used for construction of vP829 and vP658. Furthermore, the vP658 E was in an antigenically different conformation from E present in cells infected with JEV or vP829, since it reacted very poorly with D1-4G2.

The hydrophobic properties of E produced by JEV-vP829-, and vP658-infected cells were compared by using Triton X-114 phase separation experiments. Most of the radioactive E obtained from JEV- and vP829-infected cells partitioned into the detergent phase, whereas more than half of the E protein molecules synthesized in vP658-infected cells remained in the detergent phase during extraction, and no E was released from vP658-infected cells, confirming earlier studies. These results suggested that E synthesized in vP658-infected cells was improperly folded, preventing it from displaying the amphipathic properties of E produced by JEV- and vP829-infected cells. The hydrophilic forms of E found in vP658-infected cells were hypothesized to be misfolded disulfide-linked aggregates of newly synthesized viral glycoproteins. The E protein produced by vP658 is believed to be present in these types of aggregates, since most of the E protein found in vP658-infected cells did not enter the separatory gel in SDS-PAGE in the absence of reduction.

Experiments were carried out to find whether E produced by the recombinant vaccinia viruses could be incorporated into flavivirus pseudotypes. Biochemical evidence of pseudotype formation was obtained by evaluation of E in virion fractions obtained from cells coinfected with the recombinant vaccinia viruses and the 2-8 strain of JEV, which produced an E protein with an apparent higher molecular weight. Sucrose density gradient-purified virions produced by dual infection with the 2-8 strain and vP829 contained E proteins corresponding in size to E produced by the 2-8 strain and by vP829. This result indicated that E encoded by vP829 had been incorporated into the 2-8 virion, demonstrating pseudotype formation in cells coinfected with vP829. In contrast, culture fluids of cells dually infected with the 2-8 strain and vP658 showed only a single band corresponding to E encoded by the 2-8 strain, indicating that virions produced by coinfection with vP658 did not possess the E protein encoded by vP658. The absence of any vaccinia virus-derived E in the culture fluid indicated that the prM protein synthesized by 2-8 could not rescue the vaccinia virus-encoded E protein.

The production of flavivirus pseudotypes by coinfection with recombinant vaccinia viruses was further examined by using a related flavivirus, Murray Valley encephalitis virus (MVEV), and neutralization tests. Viruses harvested from cells dually infected with MVEV and vP829 could be neutralized with MAb J3-14H5 at a dose-response level identical to that for JEV. This efficient neutralization probably reflected an efficient incorporation of the overexpressed JEV E protein into the resulting viruses and may also have reflected the ability of this MAb to neutralize virus by binding to only a small number of sites on the virion, consistent with its high neutralization titer. No detectable neutralization with this JEV-specific MAb was noted in the virus harvested from cells dually infected with MVEV and vP658. These results showed that JEV E cosynthesized with prM was capable of being incorporated into the MVEV virion, whereas JEV E produced in the absence of prM was not. The finding that dual infection with vP658 failed to produce pseudotypes is consistent with the conclusion presented above that E produced in the absence of prM accumulated in an aggregated, antigenically altered form in the ER. Thus, proper folding, maturation, and assembly of E required cosynthesis with prM.

b. Protection of pigs from Japanese encephalitis viremia by inoculation of recombinant vaccinia expressing preM and E.

Recombinant vaccinia viruses were constructed expressing prM/E (vP829, vP923), and prM/E/NS1 (vP908) using the NYVAC vaccinia, a highly attenuated vector. The constructs were tested first by ip inoculation in mice that were challenged ip 3 weeks post immunization with the Beijing P3 Japanese encephalitis virus. The results of immunization and challenge are shown in Table 5 including mice immunized with a control preparation not containing Japanese encephalitis inserts (vP866). Mice immunized with the JE recombinants elicited neutralizing (N) and hemagglutination-inhibiting (HI) antibodies. The NYVAC-JEV recombinants effectively protected mice against lethal JEV challenge. These studies confirmed the ability of the two NYVAC-based recombinants (vP908 and vP923) to express JEV glycoproteins and to be immunogenic in mice.

Table 5

Immunization and JEV challenge in mice

Immunizing virus	JEV genes expressed	Antibody titer		# mice survived/ # died*
		N (90% reduction	HI	
vP829	prM/E	1:320	1:80	10/10
vP866	none	<1:10	<1:10	0/12
vP908	prM/E/NS1	1:320	1:80	11/12
vP923	prM/E	1:320	1:80	12/12

*Mice were challenged with Beijing P3 strain JEV, 3.8 x 5 dex LD50 as determined by titration using littermates.

Next, pigs were immunized. Groups of 5 Landrace cross-castrated swine of approximately 25 kg body weight were immunized by sc injection with PBS, or 8 dex pfu of parental NYVAC (vP866), or NYVAC-JEV recombinants (vP908 or vP923). Twenty-eight days after inoculation, pigs were given a second vaccinating dose administered in the same manner. No local or systemic adverse reactions were noted. Swine inoculated with NYVAC-JEV recombinants vP908 and vP923 produced N antibody averaging 1:10 to 1:40, 7 days after primary vaccination. The titers rapidly diminished and were <1:5 at 28 days. The N titers were increased after secondary inoculations, averaging 1:20 to 1:40. The time course of N antibody titers after boosting showed a rapid rise and a gradual decrease. HI values ranged from 1:4 to 1:16 in the groups vaccinated with vP908 or vP923, and unlike N antibody, HI did

not fall as dramatically. N titers determined for individual pigs in the groups vaccinated with vP908 or vP923 were similar to pool values (within one dilution of the average), showing consistent immune response to vaccination. Results from RIP analyses performed on sera collected from animals on days 0, 7, 28, 35, and 56 supported the immune response to E measured by N and HI tests. No immune responses to NS1 were detected in pigs inoculated with vP908, in contrast to previous results in mice.

The protective capacity of these NYVAC-JEV recombinant viruses was tested by challenging the immunized animals with the JEV strain B-2358/84, which was isolated from swine sera in an Aedes pseudoscutellaris cell line in Thailand and then passed one more time in this cell line and three times in C6/36 cells. JEV titers of >10 pfu/ml were detected in postchallenge sera from the PBS-inoculated control swine (4/5) and from parental virus (vP866)-inoculated swine (5/5), whereas only two of five swine inoculated with the NYVAC-JEV recombinant (vP923) exhibited viremias of >10 pfu/ml. The geometric mean of the maximum virus titers in individual swine in groups immunized with PBS and vP866 (1.2x3 dex pfu/ml) was significantly higher than the mean in groups immunized with recombinants vP908 and vP923 and 2.0 days for the two vP923-immunized swine which had titers of >10 pfu/ml. None of the five swine immunized with the NYVAC-JEV recombinant vP908 had measurable viremias of >10 pfu/ml. These results indicated that immunization with NYVAC-based JEV recombinants, vP908 or vP923, considerably reduced JEV viremia following virulent JEV challenge.

The immune response to JEV challenge was evaluated. Individual sera collected 20 days postchallenge were tested for antibodies against JEV by RIP. Swine vaccinated with NYVAC-JEV recombinant vP908 or vP923 had higher responses to E than those inoculated with PBS or vP866, indicating that the antibody reactivity to E that was present before challenge was boosted by JEV infection. Antibodies to NS3 and NS5, JEV proteins that were not expressed in the recombinant vaccinia viruses, were detected in all postchallenge sera, indicating that some level of JEV replication occurred, even in pigs that had viremias of <10 pfu/ml. Such analyses would allow one to discriminate infected from noninfected vaccinated pigs in laboratory challenge or field studies.

One pig in the parental vaccinia (vP866) group died 12 days after challenge. This animal developed a higher body temperature than any other swine during the postchallenge period and was the only animal with serum JEV titers of >1000 pfu for 3 days. JEV was not detected in postmortem brain; the animal may have died from JEV disease.

The NYVAC JEV-recombinant viruses provided safe and effective protection of swine against JEV challenge. These recombinants will next be tested in monkeys in Thailand, prior to use in human subjects.

c. Development of DEN-1, -2, -3, and -4 recombinant vaccinias expressing prM and E

The Nauru Island strain of DEN-1 was used to construct plasmids with cDNA inserts. The plasmids in turn were transfected into tissue culture cells infected with a rescuing vaccinia virus which then expressed the proteins. The vaccinias were grown in Vero cells and biosynthetic studies were done in HeLa cells. Four vaccinia recombinants were constructed that contained portions of the DEN-1 coding region. These were vP833 encoding C through NS2B, excepting that nucleotide 2894 was deleted preventing translation of NS1, NS2A, and NS2B; vP962 encoding prM through NS2B, vP1027 encoding prM and E, and vP841 encoding NS1 and NS2A.

Biochemical and immunological analyses indicated that the DEN-1 E protein expressed in HeLa cells infected by vP962 and vP1027 was identical to E produced by DEN-1 infected cells. The cell-associated forms of E precipitated by MAb D1-4G2 were the same size as E synthesized in DEN-1 infected cells. E was also released from cells infected with vP1027, and prolonged exposure of the gel used to prepare the autoradiogram showed that E was released from vP962-infected cells, but in much smaller amounts. E protein released from cells infected with vP1027 appeared to be in subviral particles, since the vP1027-encoded E co-sedimented with the recombinant JEV E protein-containing particles in sucrose density gradients. Under pulse-chase conditions only a small amount of a protein that co-migrated with E in SDS gels was precipitated from lysates of cells infected with vP833 (which encodes C, prM and E). Under conditions of continuous labelling for 8 h, cells infected with this recombinant produced a protein of the correct size which was recognized by an E-specific MAb. The poor expression of E by vP833 might reflect a difference in its stability caused by improper processing when E was co-expressed with C.

Further tests for proper expression of E by vP1027 were done by exposing the E of both DEN-1 and vP1027 viruses to cleavage with endoglycosidases. The two E's were identically glycosylated, and the extracellular forms of E contained two N-linked glycans, one immature (endo-H sensitive) and one complex (endo-H resistant, PNGase F sensitive).

NS1 proteins were expressed in cells infected with the r mbinant vaccinia viruses vP962 and vP841 that contained the complete NS1 and NS2A coding regions. Cells infected with these viruses also produced a secreted form of NS1. The secreted and cell-associated forms of NS1 protein expressed by these two viruses were indistinguishable from the DEN-1 expressed NS1 protein in terms of mobility in SDS-PAGE, and were identically glycosylated as determined by cleavage by endoglycosidases. vP833 did not induce the expression of a normal NS1 protein, consistent with the fact that a base was deleted from the NS1 coding region during vP833 donor plasmid construction. However the precipitation of a protein of higher molecular weight from vP833-infected cells by the NS1 MAb suggested that the DEN-1 polyprotein encoded by vP833 was translated in infected cells. A truncated protein reactive with a MAb to NS1 was also detected under conditions of continuous labelling.

To test the immunogenicity of DEN-1 recombinant vaccinia viruses, groups of Swiss mice were inoculated with vP1027, vP962, vP841, vP933, the parental vP452 vaccinia virus, or the DEN-1 Hawaii strain. Pooled sera collected from each group after immunization were tested for DEN-1 antibody titers. The N and HI data for these sera are shown in Table 6. Recombinant viruses vP962 and vP1027 elicited levels of N and HI antibodies comparable with those elicited by the DEN-1 virus. Recombinant vaccina vP833, vP841 and vP452 did not induce antibodies.

Table 6

Immunological response of mice to DEN-1 or recombinant vaccinia viruses

	Single inoculation					
	3 week old		6 week old		Double inoculation	
Viruses	HI	N	HI	N	HI	N
DEN-1	20*	10	20	10	40	20
vP1027	20	0	20	10	40	40
vP962	0	0	0	0	40	40
vP841	0	0	0	0	0	0
vP833	0	0	0	0	0	0
vP452	0	0	0	0	0	0

*Reciprocal of titer; 0=<20 for HI=hemagglutination-inhibition; <10 for N=neutralization.

The ability of postimmunization sera to immunoprecipitate authentic DEN-1 antigens was also tested. Sera collected after the first inoculation with DEN-1, vP962 or vP841 immunoprecipitated NS1. Sera from mice inoculated with vP452, vP833 (which contained a deleted copy of the NS1 gene) and vP1027 (which did not contain the NS1 gene) did not precipitate NS1. None of the pooled sera collected after the first inoculation, including those from animals inoculated with DEN-1, immunoprecipitated detectable amounts of E. Following the second immunization, reactions with E were obtained with sera from mice immunized with DEN-1, vP962 and vP1027 in agreement with the N and HI test results. It was concluded that vP1027 containing cDNA encoding prM and E is immunogenic in mice and is suitable for development as a component of a candidate vaccine for humans.

Based on the success with DEN-1, attempts were made to construct recombinant vaccinia viruses with prM/E cDNA of DEN-2, DEN-3, and DEN-4. Attempts were successful to purify RNA, prepare cDNA, and using the polymerase chain reaction (PCR) to amplify the prM/E gene coding region of types 2, 3, and 4 with 5' and 3' extensions tailored for efficient expression in vaccinia virus. Amplified cDNA products from all three of these genomes were inserted into the COPAK donor plasmids, generating recombinant plasmids with the DEN genes behind the vaccinia virus H6 promoter. Problems encountered in cloning the complete cDNAs of the DEN-3 and -4 genomes into the COPAK donor plasmids, apparently caused by incompatibility of the viral sequences with E. coli, were overcome by a two step cloning procedure. All three resulting plasmids were sequenced in their entirety, and in the case of DEN-2 and DEN-3, slight differences were noted between the Yale sequences and published sequences. These sequence differences were consistent with expected variations between the isolates used in this laboratory, and the isolates used to produce the published data. In the case of DEN-4, several significant discrepancies were found between the Yale sequence and the published DEN-4 sequences. However, the Yale sequence appeared to be correct and possibly the differences reflect errors in the published sequence. Repeated sequencing of the recombinant COPAK plasmids, and the sequencing of multiple, independently cloned, cDNAs has added confidence that all three donor plasmids at Yale contain authentic DEN sequences.

The cassettes containing the prM/E genes in the COPAK plasmids were inserted into the highly attenuated NYVAC strain of vaccinia virus, using the host-range complementation technique, and recombinant vaccinia viruses expressing each of the cassettes was generated and evaluated. All three recombinant viruses accurately induced the synthesis of E protein in HeLa cells. In the case of DEN-3, more intensive investigations were performed; these studies demonstrated that the recombinant vaccinia virus-encoded DEN-3 E proteins were assembled into extracellular particles that hemagglutinated goose red blood cells.

The demonstration of correct synthesis and secretion of the DEN E proteins by the four DEN recombinant vaccinia viruses strongly suggests that these will be effective vaccine candidates.

VII. A rapid diagnostic assay for eastern equine encephalomyelitis viral RNA (R. Shope in collaboration with M. Vodkin, G. McLaughlin, J. Day, and R. Novak, Purdue University and University of Florida).

The objective of this study was to develop a PCR-based assay for detecting eastern encephalitis (EE) virus in infected mosquitoes and vertebrates. The assay described here required less than 7.5 hours to process samples, reverse transcribe and amplify the cDNA, and analyze the products by gel electrophoresis.

The mosquitoes and bird tissues were collected at an emu farm in Florida during a period of EE virus circulation in 1992. The mosquitoes were processed and homogenates were stored at -70C until tested. Brain, liver, spleen and intestines of emus and pheasants were triturated and stored at -70C. Samples were inoculated into mice, and EE virus isolates were identified by mouse neutralization tests. Two positive mosquito pools and three positive emu tissues were used with controls of three negative mosquito pools and two negative pheasant tissues in a blinded trial to test the validity of the PCR-based detection.

Total RNA was extracted from each sample involving treatment with guanidium isothiocyanate-acid phenol followed by adsorption to an insoluble matrix. Total RNA was also extracted from C6/36 mosquito cells infected and mock-infected with the TenBroeck strain of EE.

Oligonucleotide primers were selected from the capsid gene of EE virus from Genbank designation EEE26SCG. They were EEE26SCG660.for AGTACGGTGATGTGCCAATG and EEE26SCG771.rev CTGTTGTTCTCATACTGCACAGC. Transcription and amplification was carried out. The reverse transcription was primed with random hexamers in a 10-ul reaction mixture. Forty repetitions of a three-step amplification cycle were used in a 50-ul reaction mixture: 94C for 1 min, 56C for 1 min, and 72C for 1 min. A final step of 72C for 5 min completed the primer extension. Reaction products or their restriction fragments were analyzed on 1-2.6% agarose gels after they were stained with 1 ug/ml of ethidium bromide.

To evaluate the efficacy of the primers, serial 10-fold dilutions of total RNA from the known infected and uninfected cell culture were amplified. A fragment of the predicted size of 112 basepairs (bp) was evident only in the infected samples. The level of sensitivity was less than 1,000 RNA molecules.

The mouse-inoculation positive bird and mosquito tissues yielded products that comigrated in gels with the 112 bp of the positive tissue culture harvest (Table 7). The negative bird and mosquito tissues did not yield the 112 bp fragment. In all the positive samples except the positive control, there was also a fragment of approximately 180 bp; this fragment, amplified from both bird tissue and mosquitoes, was also helpful in distinguishing the positive from the negative samples. Its presence was attributed either to a viral strain difference or an artifact of amplifying EE virus RNA in the background of the mosquito cell culture or of the tissue homogenates. A fragment of 70 bp was common to both positive and negative samples.

To validate that the fragment amplified from the RNA of the positive samples arose from EE virus sequences, the 112 bp band was excised from the gel, diluted, and reamplified. A BstN 1 digestion of sample 10 (Table 7), as well as of the control, yielded the expected pattern of two fragments, 62 and 50 bp in length.

Table 7

Selected field samples assayed for eastern equine encephalomyelitis virus by mouse inoculation and polymerase chain reaction (PCR)

Sample number	Contents	PCR	Mouse
1	100 *Culex nigripalpus*	-	-
2	Emu intestine tissue	+	+
3	20 *Culex erraticus*	-	-
4	30 *Aedes albopictus*	-	-
5	Emu brain tissue	+	+
6	30 *Cx. erraticus*	+	+
7	80 *Anopheles crucians*	+	+
8	Pheasant liver tissue	-	-
9	Pheasant brain tissue	-	-
10	Emu liver tissue	+	+

VIII. Evaluation of Vero cell lysate antigens for ELISA of flaviviruses (M. Ansari and R. Shope)

Vero cell lysate antigens for ELISA of flaviviruses were evaluated for sensitivity, specificity including cross-reactions and background. The evaluation was done as a comparison with the use of 4G2 MAb to capture mouse brain antigen, the routine test of the Arbovirus Research Unit. The cell lysate technique was that of Dr. Thomas Ksiazek, developed at USAMRIID, Fort Detrick. Flaviviruses were grown in Vero cells in a 150 cm2 flask until 3+ cpe was noted. The Vero cells were then scraped from the plastic, pelleted, washed 3x in borate saline buffer pH 9.0, then lysed by addition of 0.9 ml of 0.1% SDS followed by 0.1 ml of 10% Triton-X 100. Five ml borate saline buffer was added and the pellet was sonicated for 20 sec at setting 4 on a Bronson W185 sonifier. The suspension was centrifuged and the supernatant fluid constituted the antigen. The antigen was used to coat the plastic in the ELISA.

Flavivirus cell lysate antigens and the 4G2 + mouse brain antigens were used to test the sera of 17D yellow fever vaccinees, bled days 0, 14, 35, and 210 post vaccination. These sera had previously been tested by plaque reduction neutralization test, the standard for determining the sensitivity and specificity. The results are shown in Table 8.

Table 8

ELISA response of volunteers to 17D antigen expressed as the difference of OD between negative and yellow fever antigens by the standard and the Vero cell lysate method

Method	Days post vaccination	Positive	Total	Sensitivity %
Standard	14	47	52	73.1
	35	50	52	96.2
	210	47	50	94.0
Cell lysate antigen	14	29	35	82.9
	35	33	36	91.7
	210	32	36	88.9

Period t-test on comparable sera revealed no statistically significant difference (P=0.32) between the two methods at 14 days postvaccination. However, the difference between the two methods was statistically significant at 35 and 210 days postvaccination (P=0.0001). The cell lysate antigen, although less sensitive than the standard technique, still had acceptable sensitivity (about 90%).

Cross-reactions with Japanese encephalitis, St. Louis encephalitis, West Nile, dengue-1, and Zika were determined on 5 subjects. The result of OD differences greater than 0.14 was considered positive when calculating specificity. The results are summarized in Table 9.

Table 9

Evaluation of cross-reactions among flaviviruses by ELISA using the standard and cell lysate methods

Standard method - specificity

Days post vaccination	JE %	SLE %	WN %	DEN %	Zika %
14	75	100	88	88	94
35	79	100	100	94	100
210	52	100	79	75	94

Cell lysate method - specificity

14	100	88	71	100	100
35	94	100	83	100	88
210	79	79	68	94	83

At 14 and 35 days, greater cross-reactions were noted for JE by the standard ELISA than by the cell lysate method (P=0.05; P=0.03). Other differences were not significant. At 210 days, greater cross-reactions were noted for JE (P=0.05) and for DEN-1 (P=0.04); other differences were not significant.

For evaluation of background, the value of OD of WEE (control) was compared at various serum dilutions by the two methods. There were no significant differences in background.

It is concluded that if the operator is willing to give up a small amount of sensitivity, the cell lysate antigen is perfectly acceptable for use in the ELISA and has the advantage of being less expensive, sometimes more specific, and more rapid than the standard technique.

IX. Survival of Toscana virus in phlebotomine sand flies (R. Tesh, J. Lubroth, and H. Guzman)

To try to understand how phleboviruses are maintained in sand fly vectors over winter in temperate regions and extended dry periods in the tropics, experiments were done with Toscana virus in its vector, Phlebotomus perniciosus. Sand flies were reared in a colony obtained from the Istituto Superiore de Sanita, Rome and originating in Toscana Province, Italy. The ISS.Phl.3 strain of Toscana virus with a titer of 7 dex/ml was used as infected mouse brain. The virus in triturated sand flies was assayed on Vero cells. Sand fly eggs, larvae, and pupae were maintained in programmed illuminated incubators at selected temperatures, but with constant 14L:10D photoperiods and 95-100% relative humidity. Insects in control group A and infected group C were maintained at 28C. The parental females of control group B and infected group D were confined for oviposition at 25C; their eggs were held at the same temperature until the first instar larvae began hatching, when they were then transferred to 15C. To terminate diapause and

to initiate further development, fourth instar larvae held at this low temperature were sequentially transferred to 20C and 25C over a period of one or two weeks.

The parent female P. perniciosus used for studies of transovarial transmission (TOT) at different temperatures, were initially infected with Toscana virus by intrathoracic inoculation. Five days after inoculation, the 40-50 surviving flies were fed on a clean hamster to stimulate ovarian development. Six days later, the gravid females were confined in plastic containers lined with moist plaster of Paris for oviposition. After oviposition, the parent females were removed, larval food was added to the container, and the emerging F1 progeny were reared to the adult or pupal stage for virus assay. The rearing containers were examined under a stereomicroscope at regular intervals, and the insects' general development was recorded at each observation. However, because sand fly development is not very synchronous, particularly at 15C, more than one developmental stage was often present in the same container.

The ambient temperature had a marked effect on speed of development. Insects in control group A reared at summer temperature of 28C developed relatively quickly, taking 6-7 days to complete each developmental stage; the adults first appeared on day 40 and by day 55, all of the pupae had emerged. In contrast, insects in control group B reared after hatching at winter temperature of 15C, continued to feed, but were sluggish. Larval development continued slowly until the fourth instar; approximately 97 days elapsed between initial hatching and the first appearance of fourth instar larvae. The insects then entered diapause. On day 209 in an attempt to interrupt diapause, the temperature was increased to 20C, then on day 224 to 25C. Thereafter, most of the larvae pupated, and by day 249, many adults had emerged.

Offspring of infected group C were reared continuously at 28C. As shown in Table 10, Toscana virus was detected in all of the life stages sampled from this group. Thirty-three (46.5%) of 71 F1 adults tested from this group contained Toscana virus. Both males and females were infected.

Table 10

Toscana virus infection rates among various life stages of transovarially infected Phlebotomus perniciosus reared at simulated summer and winter temperatures

Life stage	Infected group C (28C)	Infected group D (15-25C)
L1	4/10*	not tested
L2	not tested	6/10
L3	2/9	2/10
L4	1/10	6/11
Pupa	3/9	8/9
Adult males	13/34	37/97
Adult females	20/37	42/64
Total adults	33/71	79/161

*number infected/number tested.

Infected group D was reared at 15C for 142 days. During this period, its rate of development paralleled that of control group B. On day 152, most of the insects in group D had reached the fourth instar, but a few third instar larvae were still present. On this date, the ambient temperature was increased to 20C, and 7 days later it was increased to 25C. The higher temperatures increased the rate of development and apparently terminated diapause, since pupae and a few adults appeared within the next 21 days.

Venereal transmission of Toscana virus in sand flies was shown; 223 F1 offspring of infected females were reared at 25C. Infection rates in the F1 adult male and female offspring were 40.6% and 56.8% respectively. Prior to virus assay, the F1 males were confined for 8 days in a cage with 120 uninfected virgin females to allow mating. After 8 days of confinement, the males were removed and tested for virus; the females were then given a blood meal and held until oviposition. After most had oviposited, the surviving females were frozen for virus assay; their offspring were also reared to adults and then tested. Toscana virus was detected in 3 (4.5%) of 66 females, indicating horizontal virus transmission (probably venereal) from the transovarially infected males. However, none of 571 offspring from eggs laid by these females was infected.

The results of these experiments are consistent with the hypothesis that Toscana virus is maintained in nature in the insect. Since TOT does not account for 100% transmission, another mechanism such as venereal transmission or amplification in a vertebrate host must be invoked to explain the success of this virus.

X. Low passage collection of arbovirus strains (R. Tesh, R. Shope, S. Tirrell)

The Unit continues to collect and archive low passage strains of arboviruses, representing different years of isolation, different geographic sites of isolation, or different host/vector sources. Several hundred strains are now in the collection. Emphasis has been given to those viruses of human and veterinary disease importance such as dengue, yellow fever, Japanese encephalitis, chikungunya, Venezuelan encephalitis, eastern encephalitis, and California group.

XI. Distribution of reagents (R. Tesh, R. Shope, and S. Tirrell)

The World Reference Center for Arboviruses distributed reagents, cells, and insects to WHO regional centers and to other laboratories throughout the world. The list of these shipments and their recipients is on pages 24-37.

CONCLUSIONS

The World Reference Center for Arboviruses received agents from the U.S. and foreign countries for characterization and identification. Sabia virus and Guanarito virus were two new arenaviruses that caused hemorrhagic fever in Brazil and Venezuela respectively. Guanarito virus was shown to be carried primarily by cotton rats. VEE variety 1E was shown for the first time to cause epizootic disease in equines, in Chiapas, Mexico. Rift Valley fever IgM was confirmed in patients with ocular disease from Egypt, the first time in 13 years this virus has returned to the Nile region. Recombinant vaccinia viruses were constructed to express proteins of Japanese encephalitis and dengue viruses. The Japanese encephalitis construct protected pigs against viremia, and the dengue recombinants are potential human vaccine candidates. The low passage arbovirus collection was augmented and reference reagents were distributed to scientists in 21 countries.

Shipments from World Reference Center for Arboviruses
(Yale Arbovirus Research Unit)
9 February 1993 - 8 December 1993

Requestor/Recipient	Item shipped	Date
Dr. Jorge Boshell Grupo de Virologia Instituto Nacional de Salud A.A. 80080 Santa fe de Bogota, Colombia	1 dengue 3 virus 1 CER cells 1 neuroblastoma (N-18)cells 1 dengue 1 monoclonal antibody 1 dengue 2 monoclonal antibody 1 Amapari polyvalent MIAF 1 Prospect Hill infected Vero E-6 cells (irradiated) 1 normal Vero E-6 cells (control) 1 dengue 3 MBA	11/30/93
Dr. C.J.Lai, Head Molecular Viral Biology Sec. Lab. of Infectious Diseases NIAID/NIH Bethesda, MD 20892	1 dengue virus (Mochizuki strain) 1 Langat virus (strain TP21)	11/29/93
Dr. James Mills Virology Division USAMRIID Fort Detrick Frederick, MD 21702-5011	1 Guanarito, MIAF 1 Junin, MIAF 1 Machupo, MIAF 1 Latino hamster immune serum 1 Parana hamster immune serum 1 Tamiami, MIAF 1 Tacaribe, MIAF 1 Flexal, MIAF 1 Amapari,MIAF 1 Pichinde,MIAF 1 Latino Virus 1 Parana virus	11/29/93
Dr. Peter W.Mason Plum Island Animal Dis.Lab. USDA Greenport, New York 11944	1 Flask LLC-Mk_2 cells	10/26/93
Dr. Duane J.Gubler Director,Div. of Vector-Borne Infectious Diseases CDC, P.O.Box 2087 Ft. Collins, CO 80525	1 Dengue-2 (INH #125271, Tachira,Venezuela) 1 Dengue-2 (INH #125270, Tachira,Venezuela) 1 Dengue-1 (INH #126397, Barinas,Venezuela) 1 Dengue-1,(INH #126183, Lara,Venezuela)	10/25/93

Requestor/Recipient	Item shipped	Date
Dr. S.D.K. Sempala Uganda Virus Research Institute P.O.BOX 49 Entebbe, Uganda	VIRUSES 1 chikungunya,strain Ross TVP2260.pass sm 16,Vero 1 1 o'nyong-nyong,strain MP 30 1 Semliki Forest,pass sml 3 Vero 2 1 Sindbis, strain UgMP 684, pass sm 7, CER 1 1 Uganda S, pass sm 24, Vero 1 1 Wesselsbron, strain SA H177, pass sm 19 1 West Nile, strain Uganda B956 pass sm 31 1 yellow fever, strain Entebbe SE7445, pass sm unknown mosq.3 1 Zika, pass sm 149 1 Bunyamwera, pass sm 47,Vero 2 1 Germiston, pass sm 18 1 Ilesha, pass sm 12 1 Tataguine, pass sm 3 1 Bwamba, pass sm 88 1 Congo, strain Ug 3010, pass sm 4 1 Thogoto, strain Sogoto, pass sm 5 1 Orungo, pass sm 6 1 Kadam, strain MP6640, pass sm 5 MOUSE IMMUNE ASCITIC FLUIDS 1 chikungunya, strain Ross 1 o nyong-nyong,strain MP 30 1 Semliki Forest 1 Sindbis 1 Uganda S 1 Wesselsbron, strain SA H177, 1 West Nile 1 yellow fever 1 Zika 1 Germiston 1 Ilesha 1 Tataguine 1 Bwamba 1 Congo 1 Thogoto 1 Orungo	10/20/93
Dr. Werner Slenczka Klinikum der Philipps-Universitat Marburg Institut fur Virologie Robert Koch Strasse 17 D-35037 Marburg,West Germany	Cell line: Anopheles stephensi,(MOS.43) Cell line: Anopheles gambiae,(MOS.55)	10/18/93

Requestor/Recipient	Item shipped	Date
Dr. Howard Caplen Government Service Div. Salk Institute P.O.Box 250 Swiftwater, PA 18370	5 Sicilian Sandfly Fever, MHAF	10/15/93
Dr. Nick Karabatsos Vector-Borne Infectious Disease Division Centers for Disease Control P.O.Box 2087 Ft. Collins, CO 80522-2087	1 Bebaru, MM2354	10/12/93
Dr. Joseph A.Mangiafico Applied Research Division USAMRIID Ft.Detrick,Frederick,MD 21701	1 Cache Valley virus, strain Tlacotalpan Mex 61D240,pass.sm9 2 Cache Valley,strain Tlacotalpan Mex 61D240 MHIAF 1 Cache Valley,virus, strain Panama BT-2368 GML207732,pass.Vero-2	10/08/93
Dr. Ricardo Galler Lab. of Molecular Virology Instituto Oswaldo Cruz Rio de Janeiro, Brazil	2 Japanese encephalitis MHIAF, strain Nakayama-NIH	10/08/93
Dr. Scott C.Weaver Dept. of Biology Univ. California-San Diego La Jolla, CA 92093	1 Lutzomyia longipalpis (LL-5) cell lines 1 Phlebotomus papatasi (PP-9) cell lines	10/06/93
Dr.Alon Warburg Lab. of Parasitic Diseases NIAID/NIH,Bldg.4/RM B-237 Bethesda, MD 20892	1 Flask Lu. longipalpis (LL-5) cell lines Larvae of Lu. longipalpis (Callejon strain)	09/00/93
Dr. Ali Zaki Virology Laboratory Dr.S.Fakeeh Hospital Palestine Road Jeddah, Saudi Arabia	5 Rift Valley fever antigen 1 Rift Valley fever mouse monoclonal antibody pool	08/03/93
Dr. Francisco Pinheiro PAHO/WHO 525 Twenty-third St.,NW Washington, DC 20037-2897	5 ampoules each of dengue antigen serotypes 1,2, 3 and 4	07/14/93

Requestor/Recipient	Item shipped	Date
Dr. H.G.Zeller Institut Pasteur B.P. 220 Dakar, Senegal	1 Wesselsbron,strain Thailand BKM-367-66, pass sm#4, Vero #1 1 Ibadan An-31956,pass.sm#4 1 Sepik,Aus Mk-7148,pass.sm#7	07/08/93
Dr. Thomas Ksiazek Special Pathogens Branch Mail Stop G14 Div. Viral & Rickettsial Dis. CDC, Atlanta, GA 30333	1 Thottapalayam virus, I-66412,sm pass.17 1 Thottapalayam MHIAF	07/07/93
Dr. Robert Swanepoel National Inst. for Virology Private Bag X4 Sandringham,2131, So.Africa	1 Pretoria,Eg Art 3089, pass sm 5 1 Thottapalayam,I-66412, pass sm 16	07/01/93
Dr. Alan D.T.Barrett Dept. of Pathology Univ. Texas Medical Branch at Galveston School of Medicine Galveston, TX 77550	1 Japanese encephalitis strain P3, sm#1 1 yellow fever,strain French neurotropic mouse passage #53,30597	06/30/93
Dr.Francisco Pinheiro PAHO/WHO 525 Twenty-third St.,NW Washington, DC 20037	1 Dengue-1,Hawaii,pass sm21 1 Dengue-2,New Guinea C, pass sm 25 1 Dengue-3, H87,pass sm 24 1 Dengue-4, H241,pass sm 18	6/29/93
Dr.Phillip Marcus Dept. Molecular & Cell Biology University of Connecticut 75 N. Eagleville Road Storrs, CT 06269-3044	1 flask Phlebotomus papatasi,(PP-9) cells 1 flask Lu. longipalpis (LL-5) cells	6/21/93
Dr. Dov Borovsky University of Florida Florida Medical Entomology Laboratory 200 9th Street, SE Vero Beach, FL 32692	100ul each of purified RNA from EE,strain TenBroeck (prototype) WE,strain MacMillan (prototype) VE,strain Everglades, (prototype VEE II) Highlands J,strain prototype Normal Vero cell extract	6/16/93
Dr. Nick Karabatsos Vector-Borne Dis.Div. Centers for Dis.Control Foothills Road Fort Collins,CO 80522-2087	1 Dengue-4, strain H241, pass sm #18	6/16/93

Requestor/Recipient	Item shipped	Date
Dr. Jorma J.Kirsi,Head Antiviral Research Section B Southern Research Institute P.O.Box 55305 Birmingham, AL 35255-5305	1 Langat TP21, pass sm#8 Vero #1	5/10/93
Dr.Vincent Deubel Institut Pasteur Unite des Arbovirus 25, rue du Dr. Roux 75724 Paris Cedex 15, France	1 Dengue-1,strain Mochizuki, pass adult mice #176, sm #4 1 human serum,Biken inacti- vated JE mb vaccine	6/08/93
Professor Max Bergoin Directeur du Laboratoire de Virologie Moleculaire Station de Recherches de Pathologie Comparee INRA-UA1184 CNRS 30380 Saint Christol-lez-Ales France	1 Culex tarsalis cell line 1 Culex quinquefasciatus cell line	6/07/93
Dr. Maja A.Sommerfelt National Centre for Research in Virology University of Bergen Bergen High Technology Centre N-5020 Bergen, Norway	1 Cocal Virus, strain TR- 40233, pass sm #8, Vero 3,TVP-563	5/29/93
Dr.Duane Gubler,Director Vector-Borne Dis.Division CDC/Foothills Road Fort Collins, CO 80522	1 Dengue-1,Mochizuki,smb, pass adult mouse 176, sm #4	5/28/93
Dr.Leonid Uryvaev D.I. Ivanovsky Inst. of Virology 16 Gamaleya Str. Russian Academy of Med.Sci. Moscow 123098 Russia	Viruses 1 WE,McMillan strain,mouse pass #14 1 Highlands J,B230,strain mouse pass #4 1 Ft Morgan,CM4-146 strain mouse pass #7 1 Whataroa,prototype,mouse pass #9/Vero pass #2 1 Babanki,Dak Y251 strain, mouse pass #4 1 Sindbis,EgAr339 strain, mouse pass #3/Vero pass #1 Mouse immune ascitic fluids 1 Western encephalitis 1 Highlands J 1 Ft Morgan 1 Whataroa 1 Sindbis	5/23/93

Requestor/Recipient	Item shipped	Date
Dr.Shan-Ing Chung Dept.of Veterinary Microbiology & Pathology Washington State University Pullman, WA 99164-7040	1 Cache Valley MIAF, strain Holden	5/20/93
Dr. Scott Weaver Dept. of Biology,0116 University of California, San Diego 9500 Gilman Drive La Jolla, CA 92093-0116	1 BeAr 18205,TVP-3454, pass sm#1,Vero #1 1 BeAr 81828,TVP-3455, pass sm#2,Vero #1 1 BeAr 300851,TVP-3458, TVP-3458,pass sm#1 1 BeAr 348998,TVP-3459, pass sm#2,Vero #1 1 BeAr 414556,TVP-3460, pass sm#3,Vero #1 1 BeAr 436087,TVP-3245, pass sm#1,Vero #1	4/23/93
Dr. Nick Karabatsos Vector-Borne Dis.Division Centers for Disease Control P.O.Box 2087 Fort Collins, CO 80522	1 Sepik virus,mouse pass#7 1 Sepik MIAF	4/12/93
Dr.Dhruba Chattopedhyay University College of Science Department of Biochemistry 35,Ballygunge Road Calcutta, 700 019, India	2 VSV-NJ, MAF	3/31/93
Scott Weaver Dept. of Biology Univ.of California,San Diego 9500 Gilman Drive La Jolla, CA 92093-0116	1 AR 18205,TVP-3454 1 AR 81828,TVP-3455 1 AR 300851,TVP-3458 1 AR 348998,TVP-3459 1 AR 414556,TVP-3460 1 AR 436087,TVP-3245	3/30/93
Dr.Michael Beach Hepatitis Branch A-33 CDC, Atlanta, GA 30333	1 vial of Sarcoma 180/TG	3/25/93
Dr. Jorge Boshell Virology Group Instituto Nacional de Salud Bogota, Colombia	1 Dengue-1 (prototype) 1 Dengue-2 " 1 Dengue-3 " 1 Dengue-4 "	3/06/93
Dr. Vasantha Kumar Harvard University Cellular & Developmental Biology 16 Divinity Avenue Cambridge, MA 02138	1 Anopheles gambiae (MOS 55) cell line	3/03/93

Requestor/Recipient	Item shipped	Date
Dr.Russell Regnery Viral & Rickettsial Zoonoses Branch CDC/MS-G13 1600 Clifton Road Atlanta, GA 30333	1 Aedes albopictus cells AA-23 line	3/03/93
Dr.Phillip Marcus Dept. Molecular & Cell Biology University of Connecticut Storrs, CT 06269	1 flask of C6/36 cells	3/01/93
Dr. Alon Warburg LPD/NIH Bethesda, MD 20892	1 Lu. longipalpis (LL-5) 1 Ph. papatasi (PP-5) cell lines	2/05/93
Dr. Barbara Israel Univ. of Wisconsin Dept. Pathobiological Sci. 2015 Linden Drive West Madison, WI 53706-1102	1 amp. Jamestown Canyon MIAF	2/09/93
Scott Weaver Dept. of Biology Univ.California/San Diego La Jolla, CA 92093-0116	1 Lu. longipalpis (LL-5) 1 Ph. papatasi (PP-5) cell lines	2/05/93
Dr. Dov Borovsky University of Florida Florida Med.Entomol.Lab 200 9th Street, SE Vero Beach, FL 32692	100 ul purified RNA solution from EE virus strain 82V-2137, Yale Vero #1	1/25/93
Dr. Armindo R.Filipe,Director Instituto Nacional de Saude Dr. Ricardo Jorge Centro de Estudos de Zoonoses 2965 Aguas de Moura, Portugal	2 lymphocytic choriomeningitis mouse serum	1/19/93
Dr. Vladimir F. Yamshchikov Emory Univ.School of Medicine 1510 Clifton Road, 3089 RRC Atlanta,GA 30322	1 original viremic serum PR 159	1/06/93

Shipments from World Reference Center for Arboviruses
(Yale Arbovirus Research Unit)

Requestor/Recipient	Item shipped	Date
Dr. E.A. Gould Institute of Virology & Environmental Microbiology Mansfield Road Oxford, OX1 3SR, England	1 Karshi virus pass,sm#5 30517,5/24/89	12/28/92
Francisco Pinheiro Pan American Health Organization 525 Twenty-third Street, NW Washington, DC 20037	2 Dengue-1, mb sucrose acetone 2 Dengue-2, mb sucrose acetone	12/28/92
Dr. Thomas Ksiazek Special Pathogens Branch Mail Stop 614 Centers for Disease Control Atlanta, GA 30333	1 BEL-11650,H517-167 1 SP H 114202	12/18/92
Dr. Ricardo Galler Instituto Oswaldo Cruz Rio de Janeiro, RJ Brazil	1 17D yellow fever vaccine, lot #6676	12/16/92
Dr.L.Terezinha Madia de Souza Diretora do Servico de Virologia Instituto Adolfo Lutz Av. Dr. Arnaldo 355 CEP 01246 Sao Paulo, SP	1 Rocio virus,SP H 34675, pass 5 1 Rocio MAF,V585-701-562 1 Ilheus virus,prototype, pass 39 1 Ilheus MAF 1 St.Louis encephalitis virus BeH203235,pass.2 1 St.Louis MAF 1 SP An71686 virus,pass.8 1 Bertioga virus, SP An 1098, pass 4 1 Bertioga MAF 1 Manzanilla virus, TR-3587, pass 5 1 Manzanilla MAF	12/14/92

Requestor/Recipient	Item shipped	Date
Dr. Terence S.Dermody Director, Lamb Center for Pediatric Research Div.of Infectious Diseases Vanderbilt Univ.Med.Center 1161 21st Avenue South Room D-7235, MCN Nashville, TN 37232-2581	1 Semliki Forest,prototype, pass.mouse 13 1 Semliki Forest, strain SA Ar2066 clone A7 [74], pass. 2 at Yale	12/11/92
Dr. Michael D.Bowen School of Public Health Dept. of Biomedical & Environ. Health Sciences Earl Warren Hall University of California Berkeley, CA 94720	1 Inkoo,strain KN-3641, pass 5 1 San Angelo, strain prototype, pass 18 1 x 0.5 ml Tahyna,strain prototype, pass 22 1 x 0.5 ml Melao,strain TRVL-9375, pass 8 1 South River,strain NJ-094F, pass 6 1 Keystone, strain prototype, pass 8 1 Serra do Navio, strain BeAr 103645, pass 3 1 x 0.5 ml Trivitattus, strain prototype, pass 4 1 Guaroa, strain prototype pass 10	12/09/92
Dr. Goro Kuno Centers for Disease Control Vector-Borne Disease Div. San Juan, Puerto Rico	1 Burma(1976),PRS-225489, TV-415,pass mosq.? 1 Singapore (1973),PRS 228758,TVP-412,pass mosq.? 1 Malaysia (1983) LN:83220, TVP-2611,pass AP-61 #2 1 Tahiti (1979) PRS 228756, TVP-413,pass mosq.? C6/36 #1 1 Malaysia (1987) LN:87390, TVP-2608, pass AP-61,#1 1 India (1966)66-4481-1, TVP-2360,pass sm17,C6/36#1	12/04/92
Dr. Gourisankar Ghosh Molecular Biophysics and Biochemistry 147 Boyer Center for Molecular Medicine Yale Univ.School of Medicine 290 Congress Avenue New Haven, CT 06510	1 Chandipura virus, strain I-653514,pass sm4 1 Chandipura MHAF,strain I-653514	12/01/92

Requestor/Recipient	Item shipped	Date
Dr. Maria Guzman Chief Virology Department Instituto Pedro Kouri Havana, Cuba	1 Dengue-4,Dominica,814669 1 Dengue-4,H241 prototype, pass. mosq.#8 1 Dengue-4,Venezuela,TVP-1975, pass AP-61 #1 C6/36 #3 1 Dengue-3,Puerto Rico,PRS 228762,TVP-416,C6/36,#1 1 Dengue-3,Burma,PRS-225489, TVP-415,C6/36 #1	11/24/92
Dr. Joel Jessee Life Technologies 8717 Grovemont Avenue Gaithersburg, MD 20877	1 Semliki Forest antibody	11/16/92
Dr. Scott Weaver Dept. of Biology,0116 Univ.California,SD 9500 Gilman Drive La Jolla,CA92093-0116	Vesicular Stomatitis virus (Indiana serotype) 1 strain BT-78 1 strain VP-98F 1 strain SJNM 1 strain L30-80 1 strain L5-85	11/11/92
Dr. C.Ben Beard Malaria Branch Centers for Disease Control Atlanta, GA 30333	2 flasks Anopheles gambiae cells (Mos.55)	10/20/92
Dr.Vincent Deubel Institut Pasteur Lab. des Arbovirus 25 rue du Dr. Roux 75724 Paris Cedex 15, Frqance	1 flask, A. pseudoscutellaris (AP-61) cell line	10/19/92
Dra.Amelia Travassos da Rosa Instituto Evandro Chagas Belem, Para, Brazil	1 LCM MIAF 2 LCM Mouse brain antigen 1 Parana hamster serum 1 Parana hamster serum 2 Parana hamster brain antigen 1 Latino hamster serum 1 Latino hamster serum 2 Latino hamster brain antigen	10/17/92
Dr. Barbara Werner State Laboratory Institute 305 South Street Jamaica Plain, MA 02130	2 Jamestown Canyon, MIAF	10/09/92

Requestor/Recipient	Item shipped	Date
Dr. Anthony James Dept. Biochemistry & Molecular Biology University of California Irvine, CA	300 adult Rhodnius prolixus (live)	10/07/92
Dr. Marius Ianconescu,Head Arbovirus Lab Ministry of Agriculture Veterinary Service & Animal Hlth P.O.Box 12 Beit Dagan 50250,Israel	1 Rift Valley fever,immune sheep serum 10 Rift Valley fever,mouse liver antigen	09/29/92
Dr.Margaret Kielian Dept. of Cell Biology Albert Einstein College of Med. Bronx, New York	1 flask of C6/36 mosquito cells	09/28/92
Dr.Ben Beard Malaria Branch Centers for Disease Control Atlanta, GA 30333	Aedes albopictus eggs Culex quinquefasciatus larvae	09/28/92
Dr.Jose M.C.Ribeiro Dept. of Entomology University of Arizona Tuscon, AZ	Lutzomyia longipalpis larvae and pupae (live)	09/28/92
Dr. Vagn Bonnevie Dept.of Clinical Chemistry Slagelse Central Hospital DK-4200 Slagelse, Denmark	1 yellow fever,17D, MIAF 1 yellow fever virus,Asibi	09/23/92
Dr.Peter B.Jahrling Virology Division USAMRIID, Ft.Detrick Frederick, MD 21701	1 amp. SP H114202 virus	10/23/92
Dr. Thomas Ksiazek Special Pathogens Branch Div.of Viral & Rickettsial Dis. Centers for Disease Control Atlanta, GA 30333	1 amp. SP H114202 virus	10/23/92
Dr.Sailen Barik,Asst.Staff Dept. of Molecular Biology, NC2-130 The Cleveland Clinic Foundation 9500 Euclid Avenue Cleveland, Ohio 44195-5069	1 Piry, MIAF 1 Rabies, MIAF 1 Isfahan, MIAF	09/16/92

Requestor/Recipient	Item shipped	Date 2
Dr. Bert Jacobs Dept. of Microbiology Arizona State University Tempe, Arizona 85287-2701	1 VSV-NJ, MIAF	09/16/92
Dr. Victor Stollar Dept. of Molecular Genetics & Microbiology Univ. of Med. & Dentistry of NJ Robert Wood Johnson Med.School 675 Hoes Lane Piscataway, NJ 08854-5635	1 flask of C6/36 mosquito cells	08/31/92
Dr. Charles H. Calisher Dept. of Microbiology Colorado State University Fort Collins, CO 80525	1 D'Aguilar, MIAF 1 Joinjakaka, MIAF 1 Koongol, MIAF 1 Mapputta, MIAF 1 Maprik, MIAF 1 Mitchell River, MIAF 1 Trubanaman, MIAF 1 Wongal,MRM 168, MIAF	08/30/92
Dr. Otavio Oliva Bio-Manguinhos/FIOCRUZ 20000 Rio de Janeiro, Brazil	1 Sindbis virus strain	08/17/92
Dr. Nick Karabatsos Centers for Disease Control Vector-Borne Diseases Division P.O.Box 2087 Fort Collins, CO 80522	1 Eyach 38 mouse immune serum	07/30/92
Dr.J.Marc Reynes Institut Pasteur de Guyane B.P. 6010 97306 Cayenne Cedex French Guiana	1 Mucambo, MIAF 1 Pixuna, MIAF 1 Mayaro, MIAF 1 VEE, MIAF 1 Ilheus, MIAF 1 Oriboca, MIAF 1 Murutucu,MIAF 1 Caraparu,MIAF 1 Guama, MIAF 1 Catu, MIAF 1 Wyeomyia, 1 Oropouche, MIAF 1 Chagres, MIAF 1 Punta Toro, MIAF	07/30/92

Requestor/Recipient	Item shipped	Date
Dr. Gerhard Dobler Abteilung fur Virologie Inst. fur Medizinische Mikro- biologie und Hygiene Technische Unniv. Munchen (Klinikum r.d. Isar) Biedersteiner Strasse 29 8000 Munchen 40, Germany	2 Eyach 38 mouse immune serum 2 Colorado tick fever,MIAF	06/30/92
Dr. Andrew K.I.Falconar London School of Hygiene & Tropical Medicine Keppel Street,London WC1E 7HT United Kingdom	1 Dengue-4,strain 814669	05/10/92
Dr.Chen Bo Quan,Director & Prof. Institute of Virology CAPM, Beijing 100052,China	1 (EEE) MIAF 1 Sindbis (strain Ar 339) MIAF	05/07/92
Dr.George French The Salk Inst.-GSD Route 611N Swiftwater,PA 18370	1 Naples virus,human un- adapted,Huddelston,strain	05/06/92
Dr.T.G.Ksiazek Special Pathogens Branch Centers for Disease Control Atlanta, GA 30333	1 Tacaribe, MIAF 1 Junin, MIAF 1 Machupo, MIAF 1 Amapari, MIAF 1 Flexal,MIAF 1 LCM,MIAF 1 Tamiami,MIAF 1 Guanarito,MIAF 1 Latino,HHS 1 Parana,HHS 1 Pichinde,MIAF	05/04/92
John R. Stephenson PHLS Centre for Applied Micro- biology & Research Porton Down, Salisbury Wiltshire SP4 OJG, U.K.	1 Dengue-3 virus,H87	05/04/92
Dr. Owen L.Wood Building 17-B US Naval Medical Research Institute, Bethesda, MD 20889	1 Langat virus,TP-21 1 Langat MAF,TP-21	04/28/92
Dr.Y.Inaba Nihon University College of Agriculture & Veterinary Medicine 1866 Kameino,Fujisawa Kanagawa 252,Japan	1 Kasba virus,IG-15534 2 Kasba MIAF, IG-15534	04/21/92

Requestor/Recipient	Item shipped	Date
Dr.Thomas Ksiazek Viral Pathogens Branch Division of Viral and Rickettsial Diseases CDC,Atlanta,GA 30333	1 Tick-borne encephalitis Far East,strain Sophyn	03/05/92

PUBLICATIONS

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Ansari, M.Z., Shope, R.E., and Malik, S. Evaluation of Vero cell lysate antigen for the ELISA of flaviviruses. J. Clin. Lab. Anal. 7:230-237, 1993.

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Brown, S.E., Gorman, B.M., Tesh, R.B., and Knudson, D.L. Coltiviruses isolated from mosquitoes collected in Indonesia. Virology 196:363-367, 1993.

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Chen, W.R., Rico-Hesse, R., and Tesh, R.B. a new genotype of Japanese encephalitis virus isolated in Indonesia. Am. J. Trop. Med. Hyg. 47:61-69, 1992.

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Konishi, E., Pincus, S., Paoletti, E., Laegreid, W.W., Shope, R.E., and Mason, P.W. A highly attenuated host-range restricted vaccinia virus strain, NYVAC, encoding the prM, E, and NS1 genes of Japanese encephalitis virus prevents JEV viremia in swine. Virology 190:454-458, 1992.

Konishi, E., Pincus, S., Paoletti, E., Shope, R.E., Burrage, T., and Mason, P.W. Mice immunized with a sub-viral partical containing the JEV M and E proteins are protected from lethal JEV infection. Virology 188:714-720, 1992.

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Pincus, S., Mason, P.W., Konishi, E., Fonseca, B.A.L., Shope, R.E., Rice, C.M., and Paoletti, E. Recombinant vaccinia virus producing the prM and E proteins of yellow fever virus protects mice from lethal yellow fever encephalitis. Virology 187:290-297, 1992.

Shope, R.E. Impact of global climate change on human health: Spread of infectious disease. In: Global Climate Change: Implications, Challenges and Mitigation Measures. S.K. Majumdar et al., eds. The Pennsylvania Academy of Science, pp. 363-370, 1992.

Shope, R.E. and Evans, A.S. Assessing geographic and transport factors, and recognition of new viruses. In: Emerging Viruses, S.S. Morse (ed.), Chap. 11, pp. 109-119, Oxford, New York, 1993.

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Tesh, R.B., and Andreadis, T.G. Infectivity and pathogenesis of iridescent virus type 22 in various insect hosts. Arch. Virol. 126:57-65, 1992.

Tesh, R.B., Lubroth, J., and Guzman, H. Simulation of arbovirus overwintering: Survival of Toscana virus (Bunyaviridae: Phlebovirus) in its natural sand fly vector, Phlebotomus perniciosus, Am. J. Trop. Med. Hyg. 47:574-581, 1992.

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Vasconcelos, P.F.C., Travassos da Rosa, A.P.A., Rodriguez, S.G., Tesh, R., Travassos da Rosa, J.F.S., and Travassos da Rosa, E.S. Infeccao humana adquirida in laboratorio causada pelo virus SPH 114202 (Arenavirus: familia Arenaviridae): Aspectos clinicos e laboratoriais. Rev. Inst. Med. Trop. Sao Paulo 35:521-525, 1993.

Vodkin, M.H., McLaughlin, G.L., Day, J.F., Shope, R.E., and Novak, R.J. A rapid diagnostic assay for eastern equine encephalomyelitis viral RNA. Am. J. Trop. Med. Hyg. 49:772-776, 1993.

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Weaver, S.C., Tesh, R.B., and Guzman, H. Ultrastructural aspects of replication of the New Jersey serotype of vesicular stomatitis virus in a suspected sand fly vector, Lutzomyia shannoni (Diptera: Psychodidae). Am. J. Trop. Med. Hyg. 46:201-210, 1992.